PHYTOCHEMICAL AND ANTIFUNGAL STUDIES ON ROOT OF *IPOMOEA SEPIARIA* KOENIG EX. ROXB.

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ABSTRACT

Plants have been one of the major sources of medicines since the beginning of human civilization. *Ipomoea sepiaria* Koenig Ex. Roxb. is a medicinal plant considered as a source plant of the classical Ayurvedic drug *Lakshmana*. The root of this plant is known for the treatment of leucorrhoea and infertility. This study was undertaken to know antifungal effect of methanolic extract of root against the fungal strain *Candida albicans* which is responsible for leucorrhoa. The inhibitory effect was assessed by agar well diffusion method. The phytochemical profile was also carried out through primary phyto-chemical screening and HPTLC analysis. The result shows that the root of *Ipomoea sepiaria* is having phyto-constituents like carbohydrates, alkaloids, glycosides, flavonoids, phenolic compounds, tannin and saponin. The methanolic extract of root was found to possess significant antifungal activity and this supports the use of root in the treatment of leucorrhoa.

KEY WORDS: *Lakshmana, Ipomoea sepiaria, Candida albicans, leucorrhoea, antifungal activity.*
INTRODUCTION

_Ipomoea sepiaria_ Koening ex. Roxb., is a source of the classical Ayurvedic medicinal plant _Lakshmana_ (Kamat SD, 2006). It is a glabrous or occasionally pubescent or hirsute, slender twining climber with a slightly thickened or tuberous perennial root (Aiyer KN et. al., 1957). The root system consists of a fairly long, somewhat thickened taproot and several slightly thinner or slender branches, arising from its base with very few wiry rootlets (Duthie JF, 1994). Leaves are simple alternate, entire, blotched with brownish patches towards the middle (Sivarajan VV, 2004). Flowers are delicate purple or white with a purple eye, along with short to long peduncles and short pedicels (Haunes HH, 1988)

In folklore practice this herb is known as a good antidote to arsenic poisoning, uterine tonic, aphrodisiac and anti-ulcer drug (Kirtikar KR and Basu BD, 1960). It is also used as diuretic, deobstruent and tonic. It is reported to be used in burning sensation, strangury, general debility and sterility in women (Prajapati ND et. al., 2003). The literatures further specify the use of root in case of diabetes (Jain SK, 1991) and constipation (Venkataswamy R et. al., 2010). In one of the Ayurvedic texts Basavarajeeyam (18th Century) it is mentioned that the root powder in the dose of 1 teaspoon is administered with rice water for leucorrhoea (Nishteswar K, 2003).

Leucorrhoea is a common condition in women of reproductive age. It refers to a whitish secretion from vagina which acts as a moisturizer and a protective coating over the vaginal wall. However, if vagina is infected by microorganisms, the normal vaginal flora become stickier, odorous and the whitish secretion becomes yellowish or greenish depending on the microbes that cause the infection. These symptoms are collectively termed as pathological leucorrhoea. Moreover, sometimes _Gardnerella vaginilis_ and _Tricomonas vaginalis_ a bacterial and a protozoal strain respectively are also known to produce leucorrhoea (Rentz AM, 1998). As _I. sepiaria_ plant is used in the treatment of leucorrhoea, it should possess some antimicrobial property. Hence in the present study antifungal activity of the root has been evaluated, also preliminary phytochemical analysis was carried out as a standardization perspective.

MATERIALS AND METHODS

Collection of plants:

The whole plant of _Ipomoea sepiaria_ (photo slides 1 & 2) was collected from the campus of Gujarat Ayurved University, Jamnagar, Gujarat in month of November. The collected samples were authenticated by the Botanical Survey of India, Office of the Scientist-'F’, Central National Herbarium, Botanic Garden, Howrah, West Bengal, specimen No.- CNH/104/2011/Tech.II/581. From the plants the roots were separated and washed properly with water and shade dried and made into a fine powder using a mechanical grinder and was sieved with mesh no. 40, and stored in an air-tight container.

Phytochemical screening:

The root powder was divided into two parts- one extracted with methanol by Soxhlet apparatus and another was extracted with water by maceration process. These extracts were subjected for the preliminary phytochemical screening for functional groups by following standard methods (Khandelwal KR, 2004).

HPTLC study:

Sample preparation:

For the HPTLC studies following samples were prepared by maceration process. The samples were titled as Track-1, Track-2 & Track-3. 
Track-1- Methanolic extract
Track-2- Chloroform extract
Track-3- Petroleum ether extract
**Mobile phase:** Toluene: Ethyl acetate: Glacial acetic acid (6.5:3.5:0.2) v/v.

**Detection:** Spray with Vanillin-sulphuric acid

**Chromatographic conditions**
- Application mode: Camag Linomat V
- Development Chamber: Camag Twin trough Chamber.
- Plates: Precoated Silica Gel GF254 Plates.
- Chamber saturation: 30 min.
- Development time: 30 min.
- Development distance: 7 cm.
- Scanner: Camag Scanner III.
- Detection: Deuterium lamp, Tungsten lamp
- Data System: Win cats software
- The developed plate was scanned to obtain densitogram in visible range from 600 nm to 800 nm with 100 nm interval.

**ANTIFUNGAL ACTIVITY EVALUATION**

**Determination of the effects:**

The antifungal activity of methanolic extract of *I. sepiaria* root against *C. albicans* was determined by using Microdilution Broth Assay (Andrews JM, 2001; Thongson C et. al., 2004) and Well Diffusion Method. Nystatin (a synthetic antifungal drug) served as positive control.

**Culturing of Candida albicans:**

A pure culture of *C. albicans* was purchased from Microbial Type Culture Center (MTCC) of Chandigarh, India (MTCC No.227) and sub culture was made using nutrient agar plate and stored in 4°C for future use.

**Macro-dilution technique for minimum inhibitory concentration (MIC) determination:**

The varying concentration of the extracts 7, 8, 25, 50, 75, 100 mg/ml were prepared and 1 ml of each concentration was added to each 9 ml of broth containing 0.1 ml of standardized test organism of *C. albicans*. The tubes were then incubated at 37°C for 24 h. After incubation period was over, the minimum inhibitory concentration was determined at the concentration in which the fungal growth was inhibited and which was clearly visible at naked eye observation (Atata RF et. al., 2003).

**Well diffusion method for determination of zone of Inhibition:**

Antifungal potential of the plant extract was tested by Agar well diffusion method (Schillinger U and Lucke F, 1989). In brief, for
fungi Malt Extract Agar plates were used. To prepare nutrient agar plates, at first 100 ml of distilled water with 2.8 g of Nutrient Agar (Himedia) was taken in a 250 ml flask. Then a cotton plug was applied to the flask and Petri plates were wrapped according to requirement. Heat was given to dissolve the constituents in the flask and kept in autoclave at 15 lbs pressure for 20 min. for sterilization. Petri plates were removed and poured the medium quickly under aseptic condition. To prepare the Malt Extract Agar plates 2 g of Malt Extract Powder (Himedia) and 2 g of Agar Agar were taken in 100 ml distilled water in a 250 ml conical flask, dissolved properly and then sterilized and Malt Agar plates were prepared.

To check the antifungal potentiality, 100 µl of fungal cultures were sprayed on their respective culture medium using a glass rod spreader. Then wells were prepared by cork borer and 50 µl of root extracts were added in each well separately. Then the plates were incubated in Incubator at 37°C. The plates were observed after 72 h of incubation. Then the zone of inhibitions by plant extracts were measured and tabulated.

**RESULTS**

The phytochemical screening of root samples for various functional groups revealed the presence of carbohydrates, alkaloids, glycosides, triterpenoids, saponins and tannin [Table 1]. In HPTLC analysis, at short UV 254 nm [Fig - 1, Fig – 2, Fig - 3) and after derivatization [Fig - 7, Fig – 8, Fig - 9), different spots were found in all three extracts i.e. methanol extract, chloroform extract and petroleum ether extract [Table-2] which indicates the presence of different phyto-components [Fig – 10, Fig – 11, Fig – 12, Fig 16]. Presence of one common Rf value (0.01) in all three samples, indicate the presence of one common component in all three extracts. At long UV 366 nm [Fig - 4, Fig – 5, Fig – 6], methanol extract, chloroform extract and petroleum ether extract showed 7, 9 and 5 spots respectively [Table-3] [Fig – 13, Fig – 14, Fig – 15, Fig 17].

**Table-1: Preliminary qualitative analysis of methanolic and water extracts of Ipomoea sepiaria root for the presence of various functional groups**

<table>
<thead>
<tr>
<th>Material</th>
<th>Reagent/Test</th>
<th>Functional group</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract</td>
<td>Kellar kiliani</td>
<td>Carbohydrates</td>
<td>Reddish brown ring</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Fehling’s solution</td>
<td>Carbohydrates</td>
<td>Red precipitate</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Dragendroff’s reagent</td>
<td>Alkaloids</td>
<td>Orange brown precipitate</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Legal’s test</td>
<td>Glycosides</td>
<td>Pink</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Brontrager’s test</td>
<td>Glycosides</td>
<td>Pink</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Alkaline reagent test</td>
<td>Flavonoids</td>
<td>Colourless</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>FeCl₃</td>
<td>Tannin</td>
<td>Green</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Ammonia test</td>
<td>Phenolic compounds</td>
<td>Yellow</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Heat test</td>
<td>Proteins</td>
<td>No coagulation</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Biuret test</td>
<td>Proteins</td>
<td>No violet colour</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Salkowski test</td>
<td>Steroids &amp; terpenoids</td>
<td>Red colour</td>
<td>Present</td>
</tr>
<tr>
<td>Water extract</td>
<td>Resin test</td>
<td>Resin</td>
<td>White precipitate</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Froth formation test</td>
<td>Saponin</td>
<td>Froth formation</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table 2: - R<sub>f</sub> value in Short UV (254 nm) of all three extracts

<table>
<thead>
<tr>
<th>NO</th>
<th>SAMPLE</th>
<th>NO. OF SPOTS</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ME</td>
<td>4</td>
<td>0.01, 0.06, 0.27, 0.77</td>
</tr>
<tr>
<td>2</td>
<td>CE</td>
<td>8</td>
<td>0.01, 0.07, 0.10, 0.13, 0.26, 0.35, 0.48, 0.77</td>
</tr>
<tr>
<td>3</td>
<td>PE</td>
<td>4</td>
<td>0.01, 0.33, 0.80, 0.91</td>
</tr>
</tbody>
</table>

ME-methanolic extract; CE-chloroform extract; PE - petrolum ether extract

Table 3: - R<sub>f</sub> value in Long UV (366 nm) of all three extracts

<table>
<thead>
<tr>
<th>SL NO</th>
<th>SAMPLE</th>
<th>NO. OF SPOTS</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ME</td>
<td>7</td>
<td>0.01, 0.08, 0.10, 0.26, 0.40, 0.80, 0.94</td>
</tr>
<tr>
<td>2</td>
<td>CE</td>
<td>9</td>
<td>0.01, 0.07, 0.10, 0.13, 0.24, 0.35, 0.43, 0.78, 0.94</td>
</tr>
<tr>
<td>3</td>
<td>PE</td>
<td>5</td>
<td>0.01, 0.30, 0.37, 0.82, 0.91</td>
</tr>
</tbody>
</table>

ME-methanolic extract; CE-chloroform extract; PE - petrolum ether extract

Table 4: Zone of inhibition of methanolic extracts of Ipomoea sepiaria root and standard

<table>
<thead>
<tr>
<th>Concentration (µg /ml)</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone of inhibition (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanolic extract of root</td>
<td>11.93 ± 0.36</td>
<td>13.35 ± 0.55</td>
<td>14.49 ± 1.19</td>
<td>15.06 ± 0.44</td>
</tr>
<tr>
<td>Nystatin</td>
<td>21</td>
<td>24</td>
<td>NA</td>
<td>25</td>
</tr>
</tbody>
</table>

Fig-1 --- Track 1 (254 nm); Fig-2 --- Track 2 (254 nm); Fig-3 --- Track 3 (254 nm)
Fig-4 --- Track 1 (366 nm); Fig-5 --- Track 2 (366 nm); Fig-6 --- Track 3 (366 nm)
Fig-7 --- Track 1 (After spraying with Vanillin sulfuric acid); Fig-8 --- Track 2 (After spraying with Vanillin sulfuric acid); Fig-9 --- Track 3 (After spraying with Vanillin sulfuric acid)

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Fig-10. 254nm peak display Track-1

Fig-11. 254nm peak display Track-2

Fig-12. 254nm peak display Track-3

Fig-13. 366nm peak display Track-1

Fig-14. 366nm peak display Track-2

Fig-15. 366nm peak display Track-3

Fig-16. Multiple Tracks (254 nm)

Fig-17. Multiple Tracks (366 nm)
From the above mentioned spectral comparison [Fig-18, Fig-19, Fig-20] some identical Rf value were found in case of all three samples i.e. 0.36, 0.86 and 0.96, which indicates presence of same phyto-component in all the three extracts.

The results of the Microdilution Broth Assay showed that the MIC of methanolic extract of root was found to be 8 µg/ml. The zone of inhibition was calculated in four concentrations i.e. 25, 50, 75, and 100 µg/ml and compared with the standard (Nystatin) [Table 4 & Photo slide -3].

DISCUSSION

Phytochemical screening in primary stages was done by performing qualitative tests. The root contains carbohydrates, alkaloids, glycosides, flavanoids, phenolic compounds, steroids, terpenoids, tannin, saponins and resin. The extract showed positive response with Kellar Kiliani test, which indicates the presence of deoxy sugar and positive response with Fehling test showed the presence of reducing sugars.

The organism tested for antifungal activity under initial screening, appeared to be susceptible to methanolic extract of root. While comparing the zone of inhibition with the standard (Nystatin 21 mm at 25 µg/ml conc.) the sample showed 11.93 mm zone of inhibition at 25 mg/ml concentration against *C. albicans*. This confirms the earlier research by Vijayan Mini *et. al.*, (2010) in which they showed that acetone extract of *Ipomoea sepiaria* has significant anti-fungal activity.
against Candida glabrata. Thus the presence of one or more phyto-constituents like carbohydrates, alkaloids, glycosides, flavonoids, phenolic compounds, tannin and saponin may be responsible for observed activity profile. However this needs further detailed research.

CONCLUSION

The methanolic extract of root of Ipomoea sepiaria is having very good antifungal activity and this supports the use of root in the treatment of leucorrhoea. Further researches are needed to explore the phyto constituents particularly responsible for the observed activity profile.

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REFERENCES


